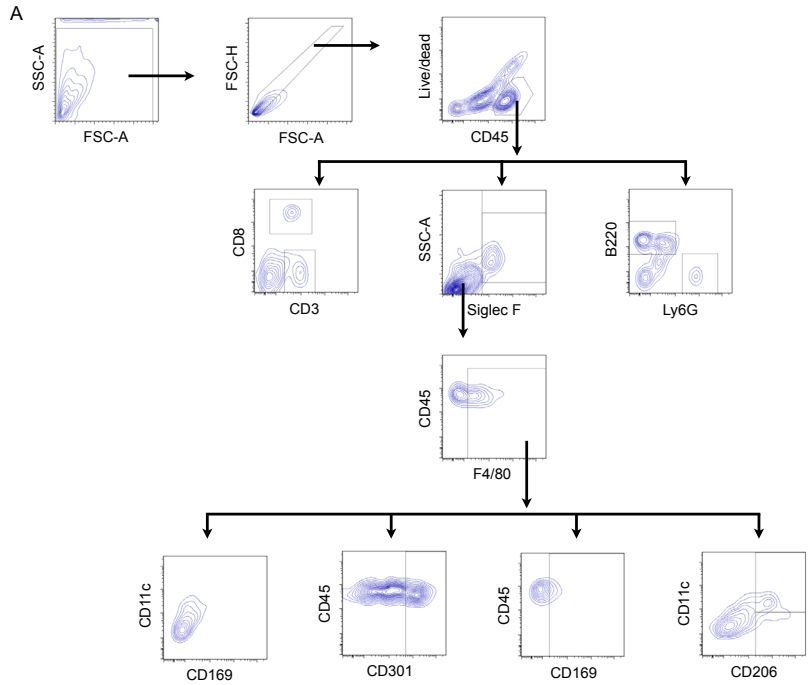
**SUPPLEMENTAL INFORMATION**

**Figure S1, related to Figure 1. Thermoneutral housing accelerates metabolic inflammation in epididymal white adipose tissue.**

(A). Gating strategy for analysis of immune cells epididymal WAT (eWAT). Mononuclear cells from eWAT were gated for forward and side-scatter (FSC/SSC), doublets, and live/dead prior to identification of to analysis of $CD3^+$ T cells, $B220^+$ B cells, Siglec $F^-F4/80^+$ macrophages, Siglec $F^+$ eosinophils, $Ly6G^+$ neutrophils. Macrophages subsets were identified as being $CD169^+$, $CD206^+$ $CD301^+$, or $CD11c^+$. (B-M) Quantification of immune cells in eWAT of mice fed normal chow (ND) or high fat diet (HFD) and housed at 22°C or 30°C (n=4-5 per diet/temperature/time point). (B and C) Numbers of $CD4^+$ cells at 22°C (B) and 30°C (C). (D and E) Total numbers of B cells at 22°C (D) and 30°C (E). (F and G) Neutrophil numbers at 22°C (F) and 30°C (G). (H and I) $CD8^+$ cells at 22°C (H) and 30°C (I). (J and K) Eosinophils at 22°C (J) and 30°C (K). (L and M) Total numbers of $CD11c^+$ $CD206^+$ cells at 22°C (L) and 30°C (M). Data are represented as mean ± SEM.


A
SSC-A
FSC-A
FSC-H
FSC-A
Live/dead
CD45
CD8
CD3
SSC-A
Siglec F
B220
Ly6G
CD45
F4/80
CD11c
CD169
CD45
CD301
CD45
CD169
CD11c
CD206

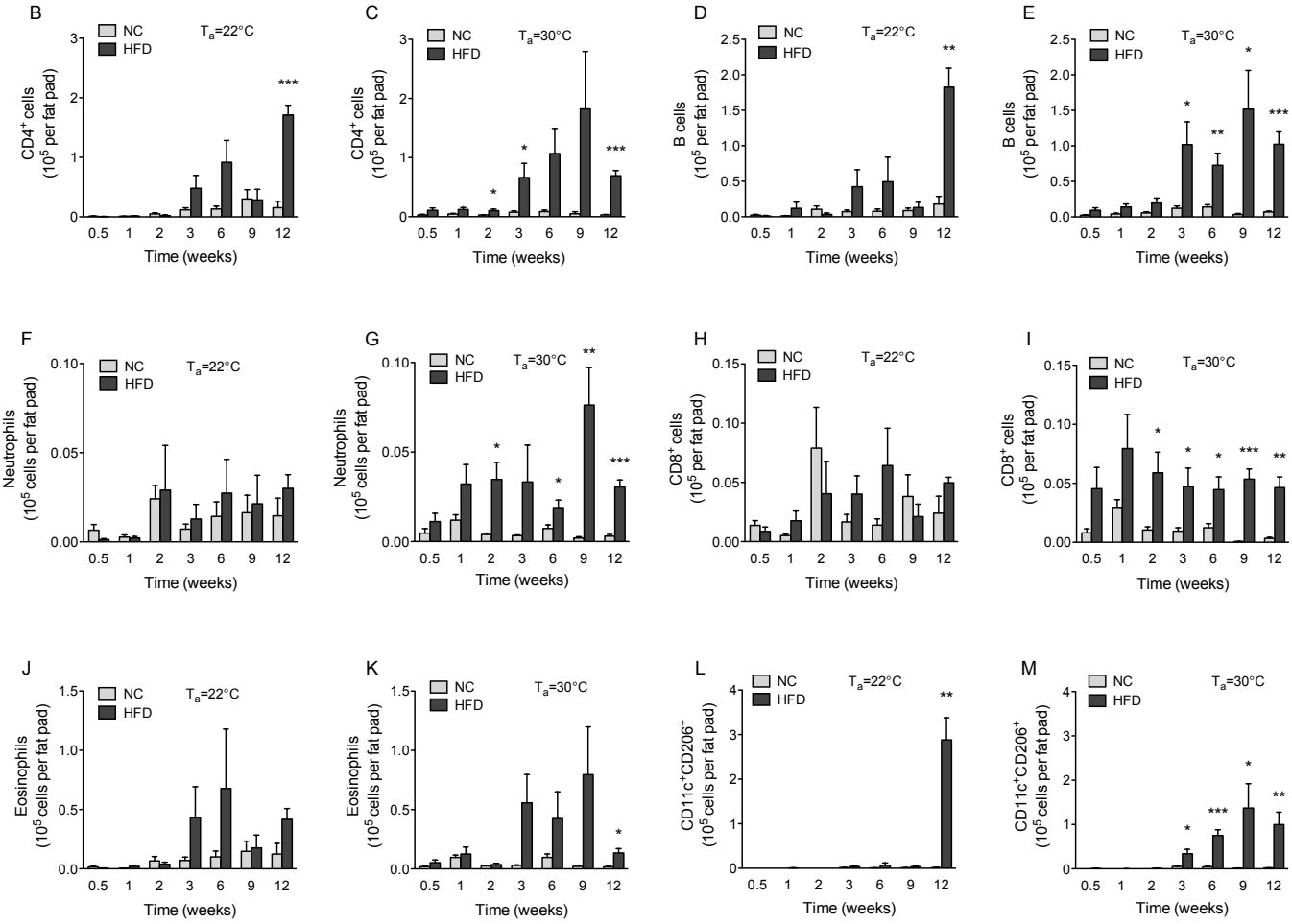

B
C
D
E
F
G
H
I
J
K
L
M
NC
HFD
Ta=22°C
Ta=30°C
CD4+ cells (10^5 per fat pad)
B cells (10^5 per fat pad)
Neutrophils (10^5 cells per fat pad)
CD8+ cells (10^5 per fat pad)
Eosinophils (10^5 cells per fat pad)
CD11c+CD206+ (10^5 cells per fat pad)
Time (weeks)
0.5 1 2 3 6 9 12

**Figure S2, related to Figure 1. Effects of thermoneutral housing on eWAT inflammation and BAT histology.**

(A-J) Quantitative RT-PCR analysis of M1 and M2 gene expression in eWAT of mice fed normal chow (ND) or high fat diet (HFD) and housed at 22°C (A-E) or 30°C (F-J). *Ccl2* (A and F), *Cd68* (B and G), *Tnf* (C and H), *Mrc1* (D and I), and *Clec10a* (E and J). Data are represented as mean ± SEM (n=4-5 per diet/temperature/time point). (K, L) Representative sections of BAT from C57BL/6J mice fed normal chow (NC) or high fat diet (HFD) and housed at $T_a$ of 22°C or 30°C were stained with hematoxylin and eosin, 100x magnification.

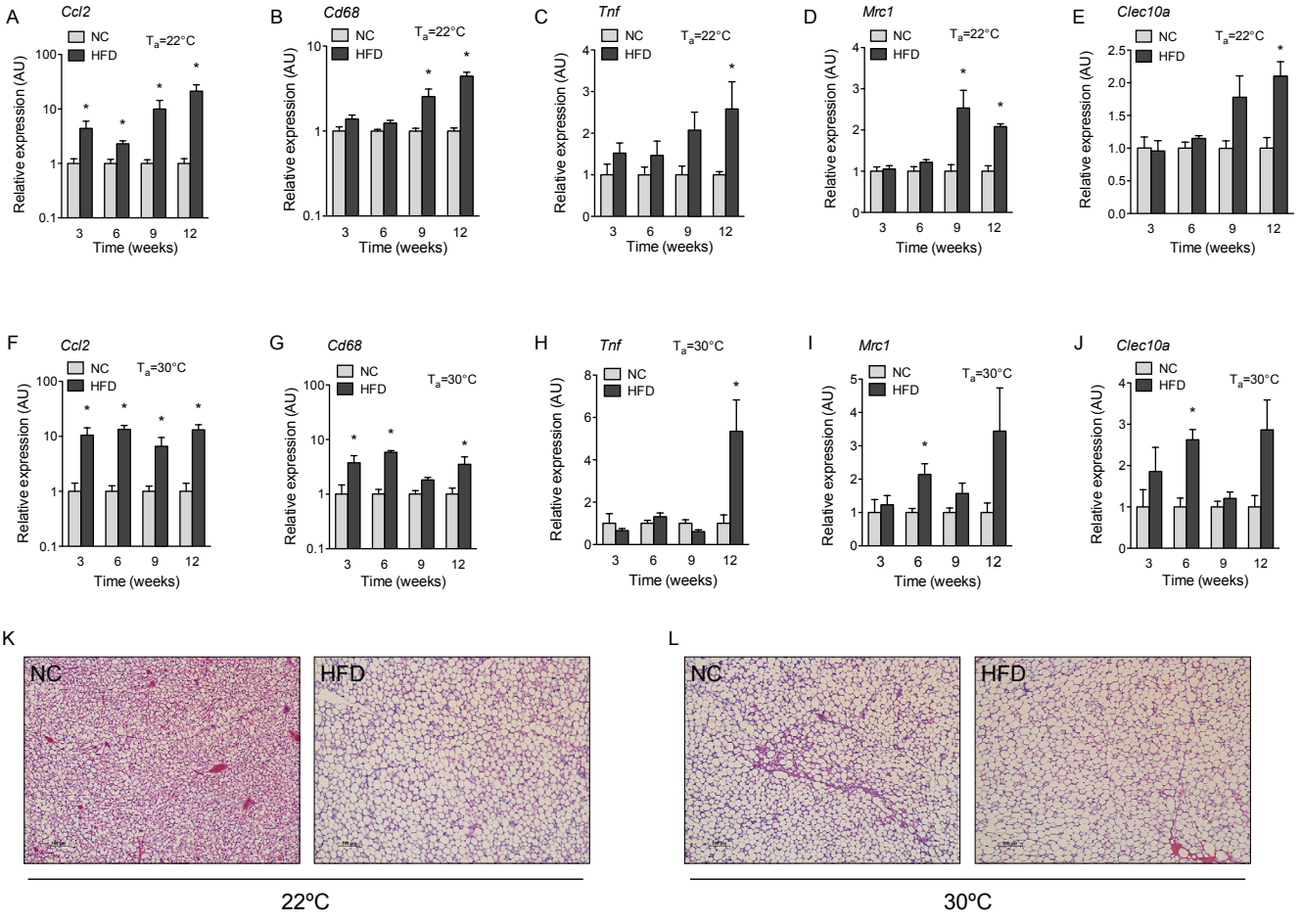


**Figure S3, related to Figure 1. Thermoneutral housing accelerates metabolic inflammation in brown adipose tissue (BAT).**

Quantification of immune cells in BAT of mice fed normal chow (ND) or high fat diet (HFD) and housed at 22°C or 30°C (n=4-5 per diet/temperature/time point). (A and B) Total numbers of $CD45^+$ cells at 22°C (A) and 30°C (B). (C and D) Macrophages at 22°C (C) and 30°C (D). (E and F) $CD11c^+$ $CD206^-$ cells at 22°C (E) and 30°C (F). (G and H) $CD169^+$ macrophages at 22°C (G) and 30°C (H). (I and J) $CD11c^-$ $CD206^+$ cells at 22°C (I) and 30°C (J). (K and L) $CD301^+$ cells at 22°C (K) and 30°C (L). (M and N) $CD4^+$ cells at 22°C (M) and 30°C (N). (O and P) B cells at 22°C (O) and 30°C (P). (Q and R) Neutrophils at 22°C (Q) and 30°C (R). (S and T) $CD8^+$ cells at 22°C (S) and 30°C (T). (U and V) Eosinophils at 22°C (U) and 30°C (V). (W and X) $CD11c^+$ $CD206^+$ cells at 22°C (W) and 30°C (X). Data are represented as mean ± SEM.

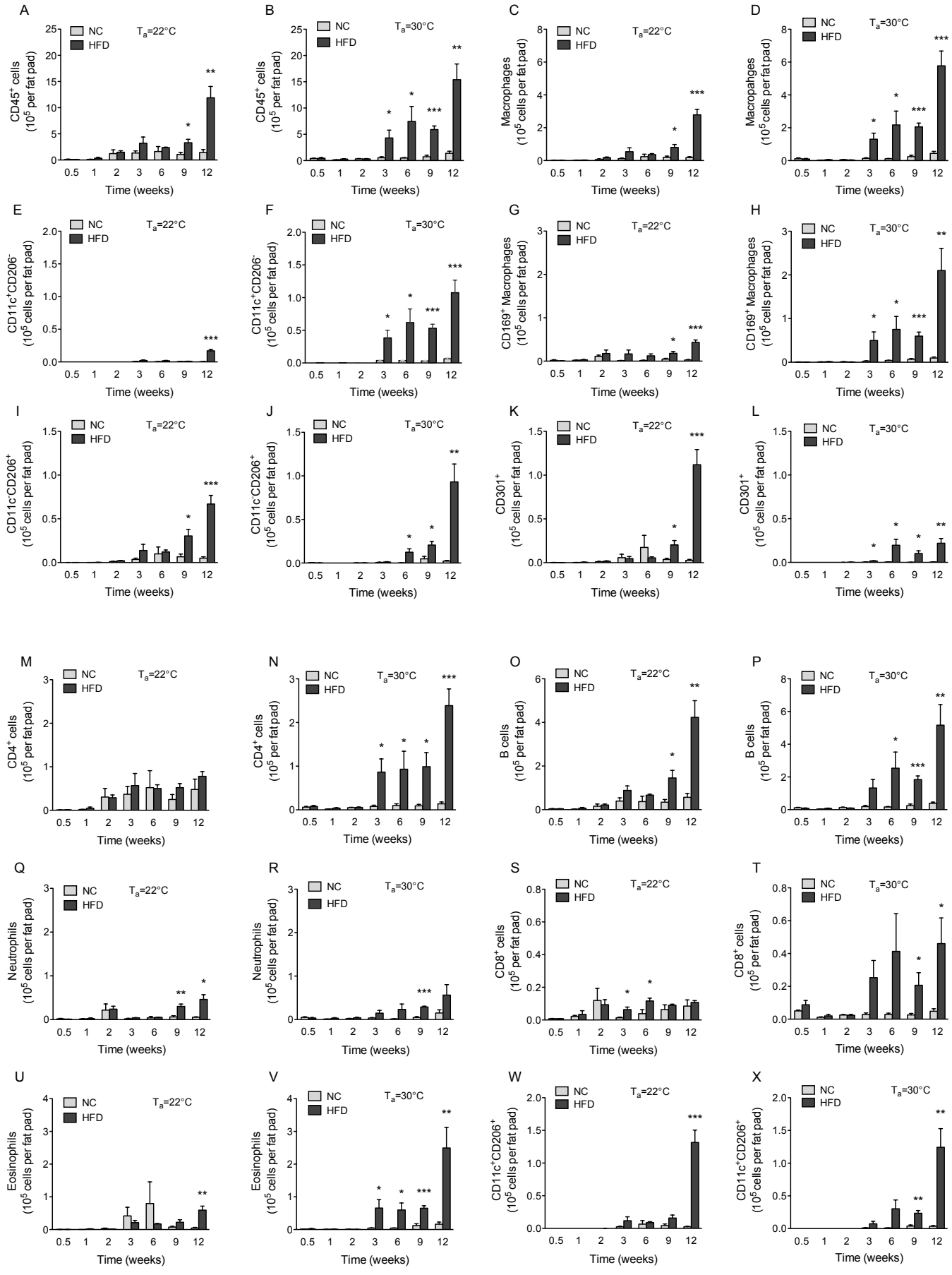

A
NC
HFD
$T_a$=22°C
CD45$^+$ cells ($10^5$ per fat pad)
Time (weeks)
B
$T_a$=30°C
C
Macrophages ($10^5$ cells per fat pad)
D
Macropahges ($10^5$ cells per fat pad)
E
CD11c$^+$CD206$^-$ ($10^5$ cells per fat pad)
F
G
CD169$^+$ Macrophages ($10^5$ cells per fat pad)
H
I
CD11c$^-$CD206$^+$ ($10^5$ cells per fat pad)
J
K
CD301$^+$ ($10^5$ cells per fat pad)
L
M
CD4$^+$ cells ($10^5$ per fat pad)
N
O
B cells ($10^5$ per fat pad)
P
Q
Neutrophils ($10^5$ cells per fat pad)
R
S
CD8$^+$ cells ($10^5$ per fat pad)
T
U
Eosinophils ($10^5$ cells per fat pad)
V
W
CD11c$^+$CD206$^+$ ($10^5$ cells per fat pad)
X
0.5 1 2 3 6 9 12

**Figure S4, related to Figure 2. Dissociation between metabolic inflammation and insulin resistance in thermoneutral mice.**

(A and B) Glucose (A) and insulin (B) tolerance tests in C57BL/6J mice fed normal chow (NC) or high fat diet (HFD) for 3-4 weeks while being housed at 22°C or 30°C (n=4-5 per diet/temperature). (C-D) Quantification of cholesterol (C) and triglycerides (D) in sera of C57BL/6J mice fed normal chow (NC) or high fat diet (HFD) for 9 weeks while being housed at 22°C or 30°C (n=5 per diet/temperature). (E-G) Metabolic effects of Western Diet (WD) on C57BL/6J mice house at 22°C or 30°C (n=7-9 per temperature). Body mass (E), glucose tolerance test performed after 6-8 h fast (F) and insulin tolerance test (G). Data are represented as mean ± SEM.

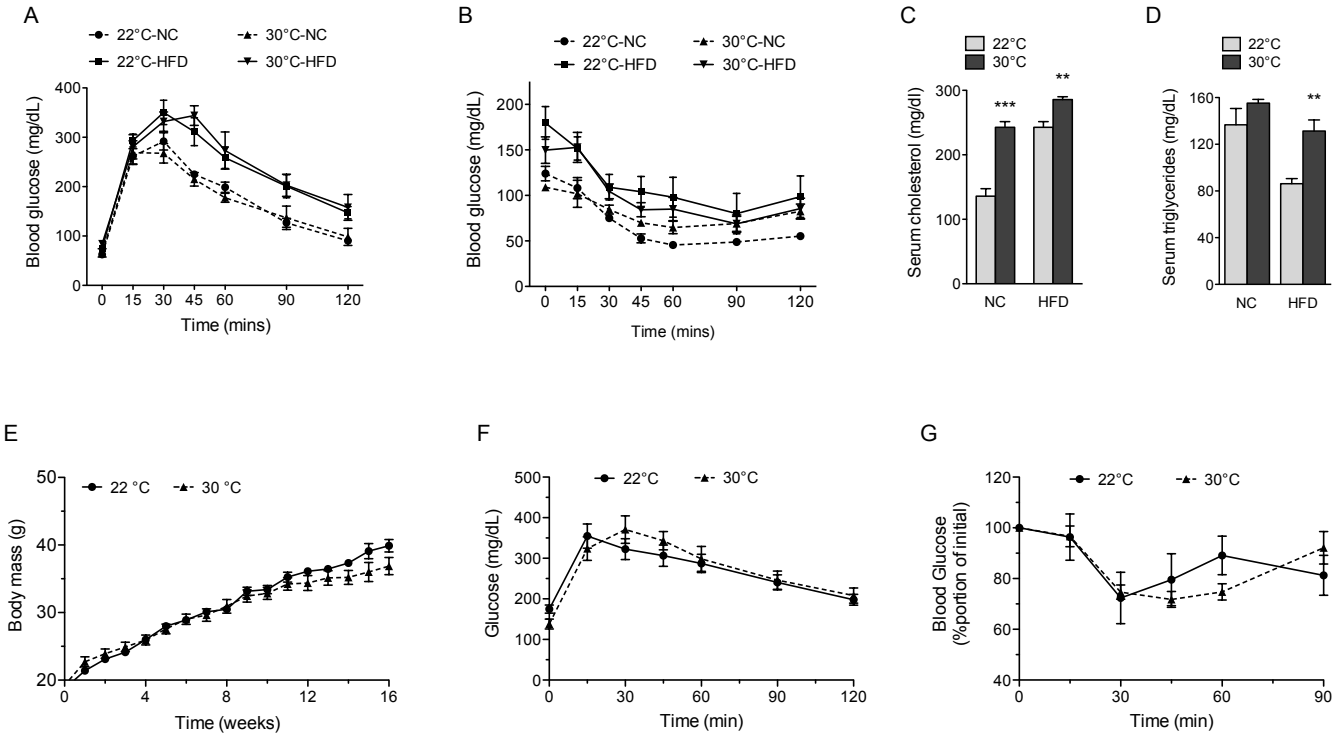
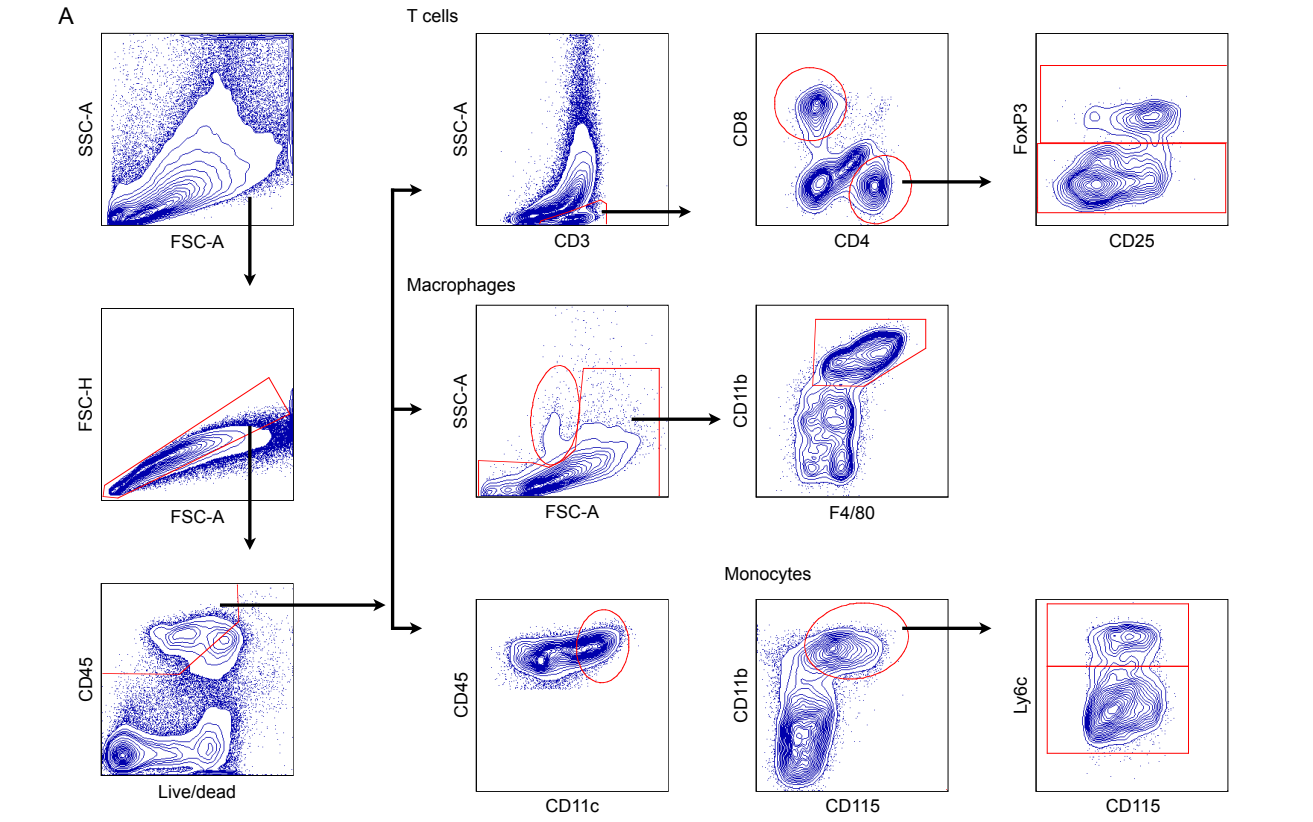
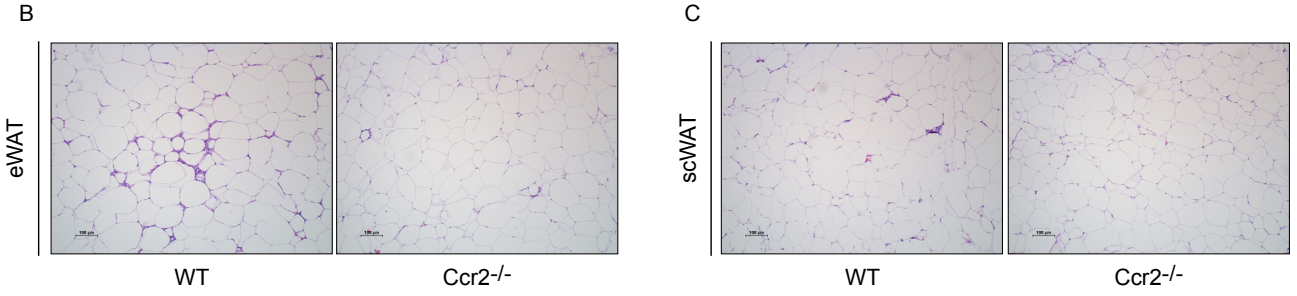

A
22°C-NC
30°C-NC
22°C-HFD
30°C-HFD
Blood glucose (mg/dL)
Time (mins)
B
22°C-NC
30°C-NC
22°C-HFD
30°C-HFD
Blood glucose (mg/dL)
Time (mins)
C
22°C
30°C
Serum cholesterol (mg/dl)
***
**
NC
HFD
D
22°C
30°C
Serum triglycerides (mg/dL)
**
NC
HFD
E
22 °C
30 °C
Body mass (g)
Time (weeks)
F
22°C
30°C
Glucose (mg/dL)
Time (min)
G
22°C
30°C
Blood Glucose (%portion of initial)
Time (min)

**Figure S5, related to Figure 3. CCR2 contributes to adipose tissue inflammation.**

(A) Gating strategy for analysis of immune cells epididymal WAT (eWAT) of WT and $Ccr2^{-/-}$ mice. Mononuclear cells from eWAT were gated for forward and side-scatter (FSC/SSC), doublets, and live/dead prior to identification of hematopoietic cells ($CD45^{+}$), $CD4^{+}$ and $CD8^{+}$ T cells, $FoxP3^{+}$ Tregs, $SSC^{lo}F4/80^{+}$ macrophages, CD11c+ cells, total monocytes, $Ly6C^{hi}$ and $Ly6C^{lo}$ monocytes. (B, C) Representative sections of eWAT (B) and scWAT (C) of WT and *Ccr2*$^{-/-}$ mice fed normal chow (NC) or high fat diet (HFD) and housed at $T_a$ of 22°C or 30°C were stained with hematoxylin and eosin, 100x magnification. (D, E) Quantification of $CD4^{+}$ (D), and $CD8^{+}$ (E) T cells in scWAT and eWAT of WT and *Ccr2*$^{-/-}$ mice fed a HFD at 30°C (n=5 per genotype). (F-H) Quantitative RT-PCR analysis of *Tnf* (F), *Itgax* (G), and *Il6* (H) in scWAT and eWAT of WT and *Ccr2*$^{-/-}$ mice fed a HFD at 30°C (n=5 per genotype). Data are represented as mean ± SEM.


A
T cells
SSC-A
FSC-A
CD3
CD8
CD4
FoxP3
CD25
Macrophages
FSC-H
CD11b
F4/80
CD45
Live/dead
Monocytes
CD11c
Ly6c
CD115


B
eWAT
WT
Ccr2-/-
C
scWAT

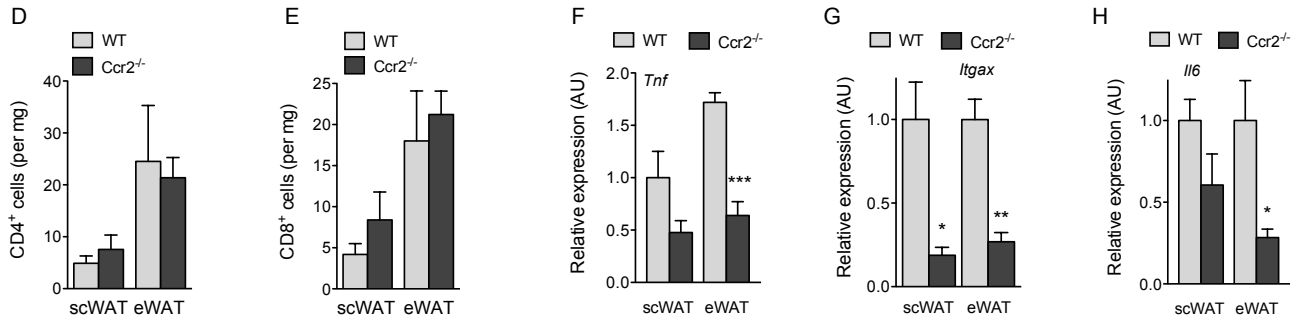
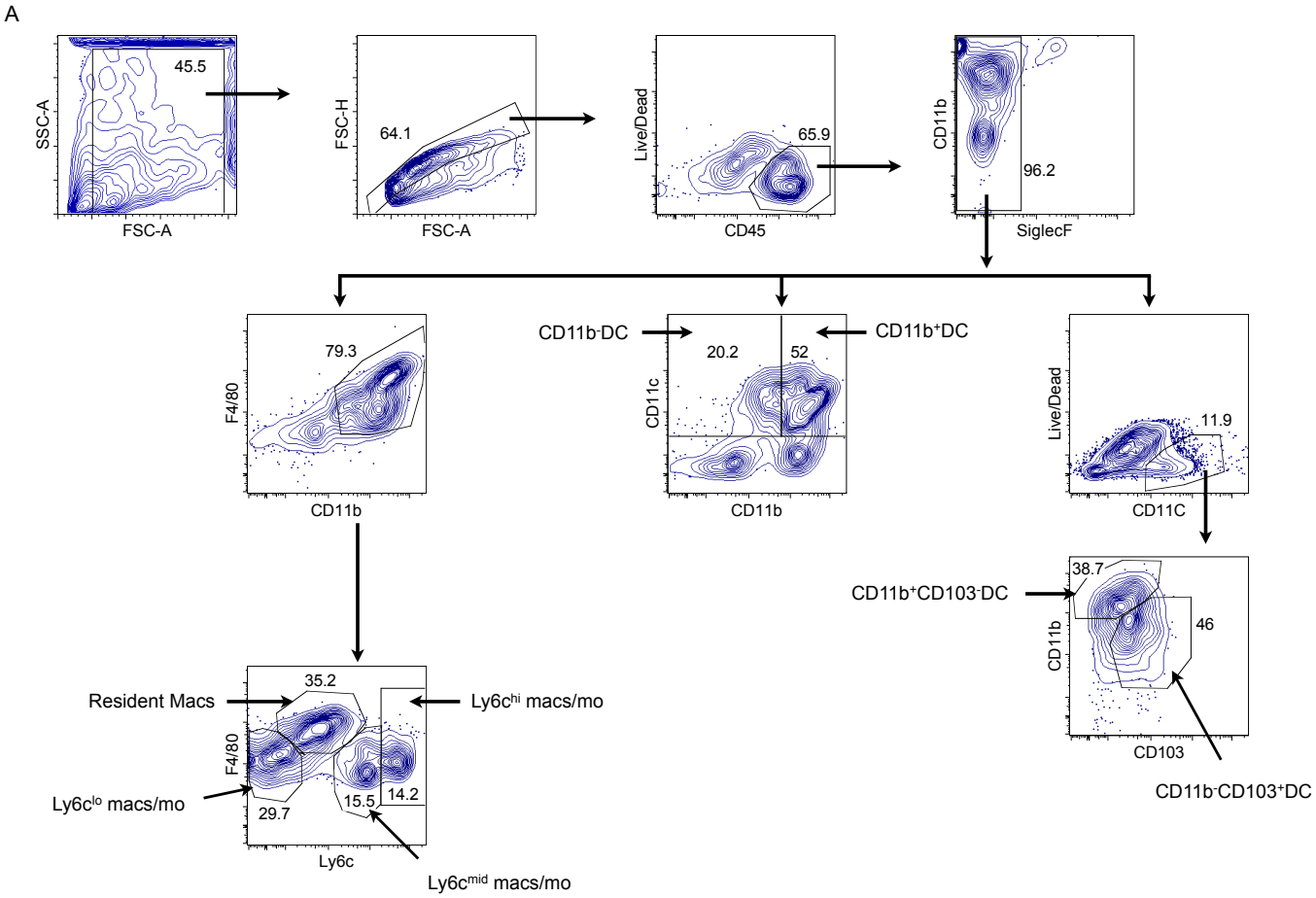

D
WT
Ccr2-/-
CD4+ cells (per mg)
scWAT eWAT
E
CD8+ cells (per mg)
F
Tnf
Relative expression (AU)
G
Itgax
H
Il6

**Figure S6, related to Figure 5. Increased immune cell infiltration into atherosclerotic lesions of thermoneutral mice.**

(A) Gating strategy for analysis of macrophages and dendritic cells in aorta of *Apoe*$^{-/-}$ mice. Mononuclear cells from aortas were gated for forward and side-scatter (FSC/SSC), doublets, and live/dead prior to identification of hematopoietic cells (CD45$^{+}$). After exclusion of eosinophils, CD11b$^{+}$SiglecF$^{-}$ cells were analyzed for different macrophage and dendritic cells subsets. Resident macrophages were defined as F4/80$^{hi}$Ly6c$^{lo\text{-}mid}$. Dendritic cell subsets were defined as CD11c$^{+}$CD11b$^{+}$CD103$^{-}$ and CD11c$^{+}$CD11b$^{-}$CD103$^{+}$ cells. (B-F) Quantification of immune cells per aorta in *Apoe*$^{-/-}$ mice fed the Western diet (WD) and housed at 22°C or 30°C (n=11-12 per group). (B) T cells, (C) CD4$^{+}$ T cells, (D) CD8$^{+}$ T cells, (E) Neutrophils, and (F) B cells. Data are represented as mean ± SEM.


A
SSC-A
FSC-A
45.5
FSC-H
FSC-A
64.1
Live/Dead
CD45
65.9
CD11b
SiglecF
96.2
F4/80
CD11b
79.3
CD11b-DC
CD11b+DC
CD11c
CD11b
20.2
52
Live/Dead
CD11C
11.9
CD11b+CD103-DC
CD11b
CD103
38.7
46
CD11b-CD103+DC
Resident Macs
Ly6chi macs/mo
Ly6clo macs/mo
Ly6cmid macs/mo
F4/80
Ly6c
35.2
29.7
15.5
14.2

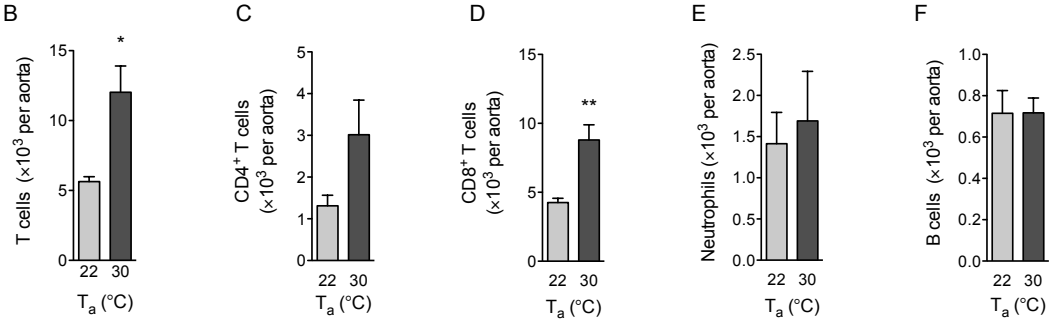

B
T cells (×10³ per aorta)
*
C
CD4+ T cells (×10³ per aorta)
D
CD8+ T cells (×10³ per aorta)
**
E
Neutrophils (×10³ per aorta)
F
B cells (×10³ per aorta)
22 30
Ta (°C)

**Figure S7, related to Figure 6. Gating scheme for analysis of immune cells in perivascular fat.**

Gaiting strategy for analysis of macrophages in perivascular fat of *Apoe*$^{-/-}$ mice fed the Western diet (WD) and housed at 22°C or 30°C.

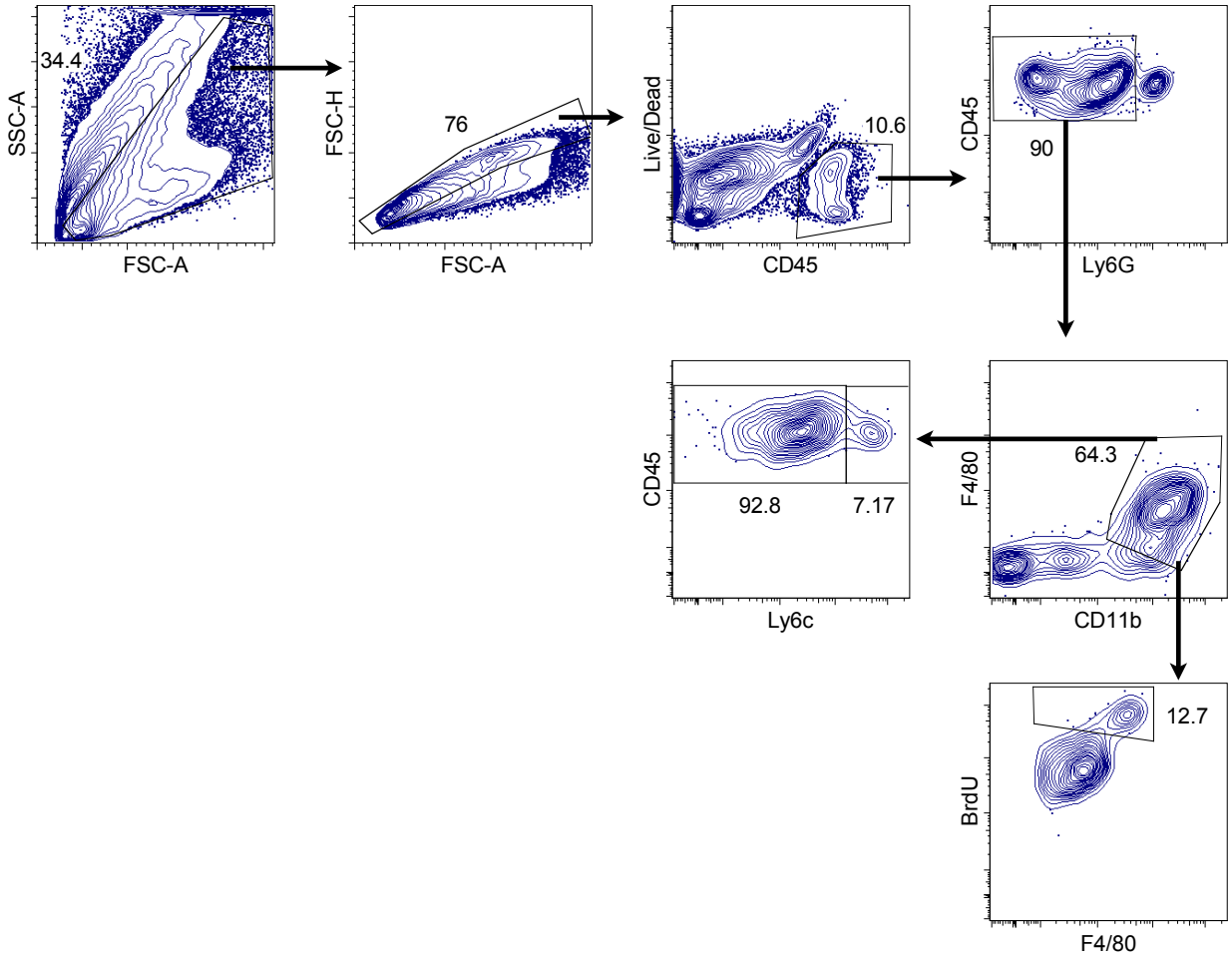


**Table S1, related to Figure 5. Analysis of immune cells in blood, bone marrow, and spleen of *Apoe*$^{-/-}$ mice housed at $T_a$ of 22°C and 30°C.**

| **Cell population** | **$T_a$=22°C** | **$T_a$=30°C** |
|---|---|---|
| | | |
| **Blood** (cell number per ml) | | |
| $CD45^+$ cells ($\times 10^6$) | 2.440 ± 0.203 | 2.948 ± 0.310 |
| Monocytes ($\times 10^6$) | 0.222 ± 0.021 | 0.316 ± 0.032* |
| $Ly6c^{hi}$ monocytes ($\times 10^6$) | 0.140 ± 0.013 | 0.164 ± 0.018 |
| $Ly6c^{lo}$ monocytes ($\times 10^6$) | 0.081 ± 0.009 | 0.152 ± 0.029* |
| $CD4^+$ T cells ($\times 10^5$) | 0.446 ± 0.087 | 0.524 ± 0.097 |
| $CD8^+$ T cells ($\times 10^5$) | 0.567 ± 0.104 | 0.631 ± 0.089 |
| B cells ($\times 10^6$) | 0.493 ± 0.058 | 0.477 ± 0.070 |
| Neutrophil ($\times 10^6$) | 0.463 ± 0.053 | 0.2682 ± 0.034** |
| | | |
| **Bone marrow** (cell number per tibia) | | |
| $CD45^+$ cells ($\times 10^6$) | 5.946 ± 0.452 | 7.396 ± 0.868 |
| $Ly6c^{hi}$ monocytes ($\times 10^6$) | 0.233 ± 0.025 | 0.372 ± 0.133 |
| $CD4^+$ T cells ($\times 10^5$) | 0.346 ± 0.054 | 0.393 ± 0.056 |
| $CD8^+$ T cells ($\times 10^5$) | 0.564 ± 0.067 | 0.592 ± 0.032 |
| B cells ($\times 10^6$) | 0.759 ± 0.204 | 0.815 ± 0.148 |
| Neutrophil ($\times 10^6$) | 2.267 ± 0.361 | 2.159 ± 0.386 |
| | | |
| **Spleen** (cell number per spleen) | | |
| $CD45^+$ cells ($\times 10^6$) | 9.647 ± 1.355 | 10.85 ± 1.012 |
| Monocytes ($\times 10^6$) | 0.570 ± 0.090 | 0.902 ± 0.110* |
| $Ly6c^{hi}$ monocytes ($\times 10^6$) | 0.134 ± 0.016 | 0.240 ± 0.038* |
| $Ly6c^{lo}$ monocytes ($\times 10^6$) | 0.408 ± 0.064 | 0.647 ± 0.079* |
| $CD4^+$ T cells ($\times 10^6$) | 1.448 ± 0.275 | 1.559 ± 0.086 |
| $CD8^+$ T cells ($\times 10^6$) | 0.743 ± 0.138 | 0.867 ± 0.066 |
| B cells ($\times 10^6$) | 3.622 ± 0.419 | 4.182 ± 0.286 |
| Neutrophil ($\times 10^6$) | 0.446 ± 0.052 | 0.336 ± 0.043 |

Quantification of immune cells in blood, bone marrow and spleen of *Apoe*$^{-/-}$ mice fed the Western diet (WD) and housed at 22°C or 30°C. Data are represented as mean ± SEM, n=6 per group. * $p<0.05$ and ** $p<0.01$ between groups.

## Supplemental Experimental Procedures

### Assessment of insulin action

Serum insulin was quantified using an insulin ELISA kit (EMD Millipore) per the manufacturer's protocol. For *in vivo* insulin signaling studies, mice were injected with insulin (0.5 $Ukg^{-1}$) through the portal vein, and liver and eWAT were harvested after 2 and 5 minutes, respectively. Tissues were lysed in modified RIPA buffer (420 mM NaCl, 1% NP-40, 0.1% SDS, 0.5% Deoxycholic acid (sodium salt), 50 mM Tris pH 7.5, and cocktail protease inhibitors) using TissueLyser II (QIAGEN). Total cellular protein (30 μg) was separated on SDS–PAGE gels, transferred to nitrocellulose membrane, and incubated with primary antibodies directed against HSP90 (1:2,000; H-114), total (1:2,000; #9272) and Ser473 phosphorylated AKT (1:2,000; clone 193H12, Cell Signaling). After incubation with secondary antibodies (1:2,000; sc-2004, Santa Cruz Biotech), proteins were detected with SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientific).

### Quantitative RT-PCR

Total RNA extracted from tissues was homogenized in TRIsure (Bioline) reagent. Reverse transcription was carried out using qScript cDNA Supermix (Quanta), and quantitative PCR reactions were performed on CFX384 real-time PCR detection system (Bio-rad). Relative expression level of mRNAs was determined using the ΔΔCt method with 36B4 or GAPDH serving as the internal reference control.

### Zymosan- induced peritonitis

6-week-old WT (C57BL6/J) and *Ccr2*$^{-/-}$ mice were housed at 22°C or acclimatized to 30°C for 2 weeks prior to initiation of experiments. Mice were injected intraperitoneally with 10μg zymosan A (Sigma-Aldrich), and the peritoneal cavity was flushed with 8ml of PBS at 4 hours (for analysis of neutrophil infiltration) or 17 hours (for analysis of monocyte infiltration). Cells were pelleted, washed, stained and subjected to flow cytometric analysis.